



## Short Report

# Clinical and molecular characterization of Diastrophic Dysplasia in the Portuguese population

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*SLC26A2*-related dysplasias encompass a spectrum of diseases: from lethal achondrogenesis type 1B (ACG1B; MIM #600972) and atelosteogenesis type 2 (AO2; MIM #256050) to classical diastrophic dysplasia (cDTD; MIM #222600) and recessive multiple epiphyseal dysplasia (rMED; MIM #226900). This study aimed at characterizing clinically, radiologically and molecularly 14 patients affected by non-lethal *SLC26A2*-related dysplasias and at evaluating genotype–phenotype correlation. Phenotypically, eight patients were classified as cDTD, four patients as rMED and two patients had an intermediate phenotype (mild DTD – mDTD, previously ‘DTD variant’). The Arg279Trp mutation was present in all patients, either in homozygosity (resulting in rMED) or in compound heterozygosity with the known severe alleles Arg178Ter or Asn425Asp (resulting in DTD) or with the mutation c.727-1G>C (causing mDTD). The ‘Finnish mutation’, c.-26+2T>C, and the p.Cys653Ser, both frequent mutations in non-Portuguese populations, were not identified in any of the patients of our cohort and are probably very rare in the Portuguese population. A targeted mutation analysis for p.Arg279Trp and p.Arg178Ter in the Portuguese population allows the identification of approximately 90% of the pathogenic alleles.

### Conflict of interest

All the authors declare not having conflicts of interest.

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*SLC26A2*-related chondrodysplasias are a group of skeletal disorders because of recessive mutations in the 'diastrophic dysplasia' (DTD) sulfate transporter (DTDST). DTD (MIM #222600) is the most known phenotype of the *SLC26A2* mutation spectrum, which spans from lethal achondrogenesis type 1B (ACG1B; MIM #600972) and atelosteogenesis type 2 (AO2; MIM #256050) to classical DTD (cDTD) and recessive multiple epiphyseal dysplasia (rMED; MIM #226900). No reliable data exist regarding the frequency of *SLC26A2*-related chondrodysplasias, but these disorders are generally believed to occur in approximately 1:100,000 (1).

Clinically, DTD is characterized by short-limbed dwarfism, normal-sized skull, cleft palate, cystic ear swelling, small chest, protuberant abdomen, spinal deformities, large joint contractures, radius dislocation, hitchhiker thumbs and clubfeet (2, 3). Radiographic findings include cervical kyphosis, bell-shaped chest, hypoplastic ilia with flat acetabula, shortened long bones with metaphyseal flaring, flat epiphyses, kyphoscoliosis with caudal reduction of interpeduncular distance, bowed radius and tibia brachydactyly, hitchhiker thumb, ulnar deviation of the fingers, shortness of the first metacarpal, delta-shaped proximal and middle phalanges (2, 3).

rMED is characterized by childhood-onset joint pain at hips and knees, mild brachydactyly, mildly shortened or normal stature and congenital clubfoot in some cases (4).

The *SLC26A2* gene (MIM #606718, locus 5q32-q33.1) consists of a 5' untranslated exon with regulatory functions and two coding exons. *SLC26A2* encodes a sulfate transporter that belongs to the family of anion exchangers known as solute carrier family 26 (SLC26) (5). The sulfate/chloride antiporter is predicted to have 12 transmembrane domains and a carboxy-terminal, cytoplasmic, moderately hydrophobic domain. This transporter is crucial for the uptake of inorganic sulfate into chondrocytes in order to maintain adequate levels of intracellular sulfate and allowing proper sulfation of the proteoglycans (6, 7). The *SLC26A2* gene is expressed not only in developing cartilage in human fetuses but also in a wide variety of other tissues (5, 7). Phenotypic manifestations of DTDST mutations are not restricted to skeleton but involve other organs containing cartilage tissue (e.g. external ears and larynx) and the ligamentous apparatus (e.g. tendons and joint capsules).

Impaired activity of the sulfate transporter in chondrocytes and fibroblasts causes intracellular sulfate depletion, which leads to insufficiently sulfated proteoglycans (8, 9). Hence, cartilage

has reduced total sulfate content and contains undersulfated glycosaminoglycans (9–11), which compromise enchondral bone formation (7, 12).

Recent studies in transgenic mice showed that proteoglycans are not only structural components of cartilage architecture, but also play a dynamic role in the regulation of chondrocyte growth and differentiation (13).

The four most common *SLC26A2* mutations are p.Arg279Trp, c.-26+2T>C (also known as 'Finnish allele'), p.Arg178Ter and p.Cys653Ser. These mutations account for 70% of disease alleles (66% in DTD cases and 90% in rMED cases) and can be detected by restriction enzyme digestion and gel electrophoresis screening. Sequence analysis may detect rarer mutations (1, 14).

The predicted severity of the mutations can be correlated with the residual activities of the sulfate transporter and the severity of the phenotypes (10–12, 14–17). In humans, correlations between *SLC26A2* mutations and clinical phenotypes have been described: 'severe' mutations (premature truncation of the protein or mutations in transmembrane domains) lead to the absence/minimal residual activity of the sulfate transporter and homozygosity or compound heterozygosity results in a severe phenotype (ACG1B) (18). 'Mild' mutations (missense mutation in non-transmembrane domain, cytoplasmic tail of the protein or in the regulatory 5'-flanking region) produce a protein with significant residual activity, which leads to either rMED ('mild' mutation in homozygosity) or to a mild form of DTD (in association with a 'severe'/'moderate' mutation). Hence, dealing with a recessive disorder, the milder mutation tends to 'rescue' the effect of a severe mutation (14, 19).

In the current literature, all reports of large series of patients with DTD are from Finland, where most patients present homozygosity for the 'Finnish mutation'. However, these conclusions may not apply to other populations. This study aimed at recruiting a cohort of DTD/rMED patients from Portugal and at studying their clinical and molecular aspects.

## Patients and methods

### Patients

The four main Portuguese Services of Medical Genetics and four Orthopedics Services were contacted in order to recruit, at a national level, patients already diagnosed as DTD or rMED or suspected of being affected by these disorders. We invited the physicians in those services to

fill in a screening protocol that we designed with a list of signs and symptoms compatible with *SLC26A2*-related, non-lethal, skeletal dysplasias (limb shortening, normal-sized skull, trunk shortening, hitchhiker thumbs, small chest, protuberant abdomen, contractures of large joints, dislocation of the radius, cleft palate, cystic ear swelling in the neonatal period, ulnar deviation of the fingers, gap between the first and second toes, clubfoot and flat hemangiomas of the forehead). Fourteen patients were selected for detailed clinical examination and X-ray review. All patients were referred by the Medical Genetics Services; eight of them were already in follow-up in the Orthopedics Services (and six patients were not being followed in Orthopedics).

A clinical protocol concerning family and personal history and tailored physical exam was performed in all patients. Heights were plotted against charts developed from Finnish DTD patients (20). Skeletal survey was requested and analyzed in all of them.

The clinical protocol, the photos and the X-rays were reviewed by the authors with the largest experience on these disorders (ASF and LB), who confirmed the diagnosis. Thereafter, the selected patients were screened for *SLC26A2* (DTDST) mutations. All patients with the clinical diagnosis made by us were mutation positive.

#### Molecular analysis

The *SLC26A2* gene was amplified by polymerase chain reaction (PCR) and screened for the four most common mutations by restriction enzyme digestion and gel electrophoresis. Subsequently, selective fragments of the gene were analyzed by bi-directional fluorescent direct sequencing. Results were confirmed in a second amplification product. If both mutations were not identified, the entire gene was then amplified by PCR and analyzed by bi-directional fluorescent direct sequencing. Mutations have been classified according to GenBank accession number U14528. Nucleotide 1 has been counted as the first nucleotide of the sequence, with nucleotide 28 being the first nucleotide of the translation initiation codon.

## Results

#### Clinical characterization

The clinical data of the patients can be consulted in Table 1. Fourteen patients were recruited, nine males and five females, with ages varying between 2 and 40 years (17 years on average). Phenotypically, eight patients were classified as cDTD,

four patients as rMED and two patients presented with an intermediate phenotype between DTD and rMED (mild DTD, mDTD). All patients were caucasian and from ancient Portuguese descent. No history of consanguinity was reported in any of the patients.

In order to accurately evaluate the growth of the patients, their height was plotted against graphics designed specifically for DTD patients (20). However, these graphics have been designed based on a population of Finnish patients most of whom are homozygous for the 'Finnish' mutation, which is mild. Moreover, the average height of the background Portuguese population is lower than that of northern Europeans. Patients with cDTD in our series were indeed under the 25th centile (some of them even under the 10th centile). Patients with mDTD were around the 25th centile and patients with rMED were above the 50th centile.

Concerning the craniofacial abnormalities, facial dysmorphism (high forehead, forehead hemangioma, downslanting and short palpebral fissures, long nose with hypoplastic alae nasi and small mouth) was only detected in cDTD. Patients with both cDTD and mDTD consistently presented dysplastic ears and hoarse voice (possible because of changes in the laryngeal cartilage structures). Cleft palate was more common in cDTD (37.5% of the patients) but, interestingly, one patient (P13) of the rMED group presented bifid uvula.

All patients with DTD presented spine deformities, the most common being cervical kyphosis. Arthrosis of the hips was a common complaint in patients older than 25 years.

Limb shortening, bowed diaphyses and contractures of large joints were present in all patients with DTD, while only occasionally in patients with rMED. The typical hand findings were striking in cDTD but subtle in the mDTD. Clubfeet and shortened Achilles tendons were present in almost all patients (DTD and rMED). However, the youngest and also least affected patient of this cohort only presented limitation of dorsiflexion of the feet.

Most patients underwent orthopedic surgery. However, despite multiple surgeries of the feet, the outcome was poor in most of them. Two patients had surgery for spinal cord decompression because of severe kyphoscoliosis. It has been recently shown that early surgical intervention might be a better option in many patients with DTD as brace treatment does not prevent progression of spinal deformities (21). Concerning quality of life, all patients had limitations; the degree of autonomy could not be predicted by their genotype. In fact, different outcomes were observed in patients with the same genotype. Possible explanations

Table 1. Clinical, radiological and molecular characterization of the 14 patients

Patient	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Gender	F	F	M	M	M	M	F	M	M	F	M	M	M	F
Age (years)	15	12	24	37	40	26	36	6	12	12	6	7	8	2
Family history	SA <sup>c</sup>	TOP	TOP	—	SA (6)	SA (5)	—	—	—	—	—	—	SA (14)	SA (13)
Height (centile) <sup>a</sup>	<10	10	<10	<10	<10	25	<10	10–25	25	25	90	75	50–75	50–75
Craniofacial														
Dysmorphism	+	+	+	+	—	—	—	+	—	—	—	—	—	—
Cleft palate	—	+	+	—	—	—	+	—	—	—	—	—	+	—
Deafness	+	+	—	—	—	—	—	—	—	+	—	—	—	—
Dysplastic ear	+	+	+	+	+	+	+	+	+	+	—	—	—	—
Hoarse voice	+	+	+	—	+	+	+	+	+	+	—	+	—	—
Spine														
Cervical kyphosis <sup>b</sup>	+	+	+	+	+	+	+	—	+	+	—	—	—	—
Lumbar lordosis <sup>b</sup>	+	—	—	+	—	—	+	+	—	+	+	+	—	—
Scoliosis <sup>b</sup>	+	+	—	+	+	—	+	—	—	+	—	—	—	—
Arthrosis of hip	—	—	—	+	+	+	+	—	—	+	—	—	—	—
Limb shortening <sup>b</sup>	+	+	+	+	+	+	+	+	+	+	+	—	+	+
Bowed diaphysis <sup>b</sup>	+	+	+	+	+	+	+	+	+	+	+	—	—	—
Contractures	+	+	+	+	+	+	+	+	+	+	—	—	—	—
Hands														
Brachydactyly <sup>b</sup>	+	+	+	+	+	+	+	+	+	+	—	—	+	+
Hitchhiker thumbs <sup>b</sup>	+	+	+	+	+	+	+	+	—	—	—	—	—	—
Fingers: ulnar deviation <sup>b</sup>	+	+	+	+	+	+	+	+	+	+	+	—	+	+
Phalangeal synostosis <sup>b</sup>	+	+	+	+	+	+	+	+	+	—	—	—	—	—
Pretibial dimples	+	—	+	—	—	—	+	—	+	—	—	—	—	—
Feet														
Clubfoot <sup>b</sup>	+	+	+	+	+	+	+	+	+	+	+	+	+	—
Shortened tendons	+	+	+	+	+	+	+	+	+	+	+	—	+	—
Treatments														
Physiotherapy	+	+	+	+	+	+	+	+	+	+	+	+	+	—
Surgery: clubfoot/tendon	+	+	+	+	+	+	+	+	+	+	+	+	+	—
Surgery of spine	+	—	—	—	—	—	+	—	—	—	—	—	—	—
Outcome	P	P	P	A	P	A	A	A	P	A	G	G	P	G
Phenotype	cDTD	cDTD	cDTD	cDTD	cDTD	cDTD	cDTD	cDTD	mDTD	mDTD	rMED	rMED	rMED	rMED
Molecular analysis of	R279W	R279W	R279W	R279W	R279W	R279W	R279W	R279W	R279W	R279W	R279W	R279W	R279W	R279W
SLC26A2 gene	R178X	R178X	R178X	R178X	R178X	R178X	N425D	R178X	c.727-1G>C	c.727-1G>C	R279W	R279W	R279W	R279W

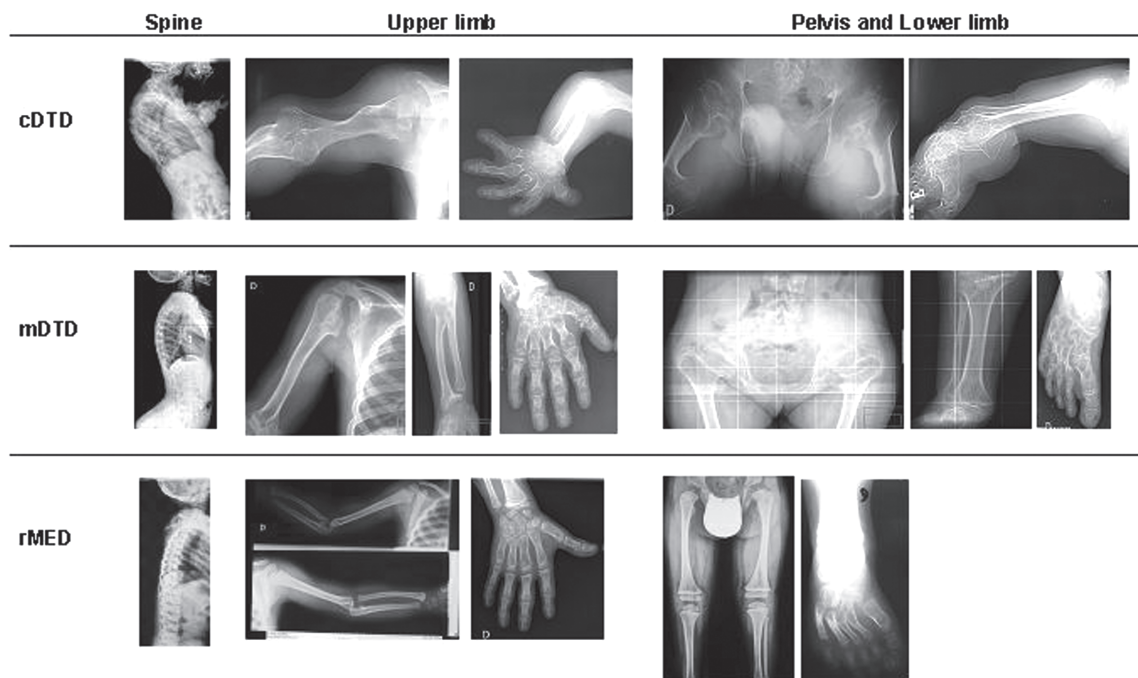
A, acceptable (autonomous ambulation); cDTD, classical diastrophic dysplasia; F, female; G, good (autonomous in everyday life activities); M, male; mDTD, mild diastrophic dysplasia; P, poor (no ambulation); rMED, recessive multiple epiphyseal dysplasia; SA, sib affected (in brackets is the attributed number of the sibling in our cohort); TOP, termination of pregnancy of affected fetus; N425D = Asn425Asp; R178X = Arg178Ter; R279W = Arg 279Trp.

<sup>a</sup>According to the growth curves proposed by Mäkitie et al. (20).

<sup>b</sup>Features better appreciated in the X-Rays.

<sup>c</sup>Sib-affected deceased in neonatal period.





**Fig. 1.** Radiological features. Patients with classical diastrophic dysplasia (cDTD) present kyphoscoliosis, upper and lower limb show shortened long bones with some metaphyseal flaring, bowed radius and tibia, hands with hitchhiker thumb with ulnar deviation of the fingers, hypoplastic ilia with flat acetabula and clubfeet. Patients with mild diastrophic dysplasia (mDTD) present similar but milder radiological presentation than cDTD. Patients with recessive multiple epiphyseal dysplasia show moderately shortened long bones, hypoplastic ilia with flat acetabula and club feet.

could subside in the genetic background, medical management, treatment options/timing/availability and family support.

Concerning the X-Ray analysis (Fig. 1, Table 1), we verified that all patients with cDTD presented with skull of normal size, cervical kyphosis, shortened long bones with some metaphyseal flaring, bowed radius and tibia, abnormalities of the hands (with hitchhiker thumb with ulnar deviation of the fingers being the most striking) and hypoplastic ilia with flat acetabula. mDTD patients had similar but milder radiological presentation. Patients with rMED consistently presented with skull of normal, moderately shortened long bones, hypoplastic with flat acetabula ilia and club feet.

#### Molecular characterization

Molecular characterization of the patients is presented in Table 1. Four known mutations were identified in our patients: c.559C>T (p.Arg178 Ter), c.727-1G>C (previously described as g.IVS 2-1G>C), c.862C>T (p.Arg279Trp) and c.1300 A>G (p.Asn425Asp) (Fig. 2).

#### Discussion

This paper reports the largest series of DTD patients in a non-Finnish population. All patients

had short stature; however, the severity was decreasing from cDTD to mDTD and to rMED. Patients with DTD (both classical and mild) consistently presented dysplastic ear and hoarse voice, cervical kyphosis, limb shortening, bowed diaphysis, contractures of the large joints, clubfeet and shortened Achilles tendons. Hand findings were more severe in cDTD than in mDTD and hitchhiker thumbs were only identified in cDTD. Patients with rMED commonly had clubfeet and subtle hand finding (moderate brachydactyly with ulnar deviation of the second finger).

Although specific features can be attributed to the different phenotypes, it is interesting to note that there is also an overlap between the different phenotypes. This is shown here between DTD and rMED (Fig. 3), but it is also true for the other *SLC26A6*-related dysplasias (between ACG1B and AO2 and between AO2 and DTD) (22–26). The spectrum of *SLC26A2*-related dysplasias can hence be described as a continuum, while also continuously expanding (27). Apart from the combination of different mutations in the *SLC26A2* gene, the variations in phenotypes could be due to modifiers of the *SLC26A2* gene, to variations of other genes involved in sulfate metabolism (6, 7, 13), as well as to the genetic background and environment. The significant role of these factors can be



Fig. 2. The sulfate transporter encoded by the *SCL26A2* gene is represented as a horizontal bar; the 12 putative transmembrane domains are shaded dark gray and the cytoplasmic domains are shaded light gray. The mutations identified in our patients are shown: Arg178Ter – diamond; c.727-1G>C – arrow; Arg279Trp – circle; and Asn425Asp – triangle.

best appreciated in the two pairs of siblings of the cohort (patients 5 and 6 and patients 13 and 14) with slightly different presentation and significantly different outcome while carrying the same mutations. The older pair of sibs (patients 5 and 6), presenting with cDTD, have overlapping phenotypes except for the presence of scoliosis and severe clubfeet in the eldest brother that has limited his ambulation. Both brothers were surgically interventioned multiple times (five times the eldest and seven times the youngest). The other pair of sibs, affected with rMED, had more significantly different phenotypes: besides a bifid uvula, the elder brother had contracture of knees and clubfeet that, together, restrained his ambulation. On the contrary, his sister was very mildly affected and the diagnosis could be suspected only by family history and careful physical exam.

Four known mutations were identified in our patients: c.559C>T (p.Arg178Ter), c.727-1G>C, c.862C>T (p.Arg279Trp) and c.1300A>G (p.Asn425Asp). The mutations p.Arg178Ter and p.Asn425Asp are localized in transmembrane domains (Fig. 1) and hence are predicted to cause a severe

disruption of the sulfate transporter function. Mutation p.Arg178Ter is a recurrent mutation that results in ACG1B when homozygosity or compounded with another severe mutation. Indeed, functional studies have showed that sulfate transporters bearing the p.Arg178X and p.Asn425Asp mutations have residual activity of <10% compared to the wild-type transporter (28). The missense mutation p.Arg279Trp occurs in an extracellular loop. Indeed, functional analysis showed that the DTDST transporters bearing that mutation had 39–62% activity compared to the wild type (16). The c.727-1G>C, very rare in non-Portuguese populations, is thought to interfere with the splicing and has been described as pathogenic (29). The presence of this mutation in 2 of 14 unrelated Portuguese patients may indicate that this variant is more common in the Portuguese population.

As previously reported, Arg279Trp, which is a ‘mild’ mutation (with significant sulfate transporter function) in homozygosity, causes the phenotype of rMED, but the association of this ‘mild’ mutation with a null mutation (Arg178Ter) or a missense mutation in the transmembrane domain

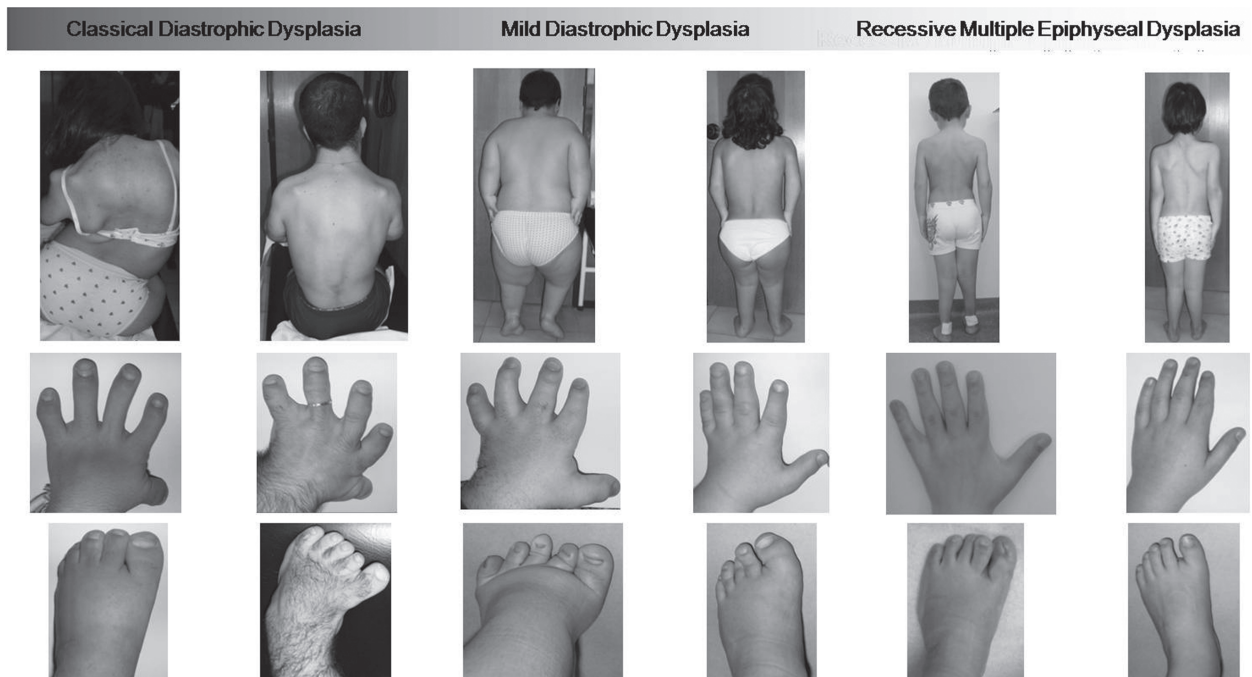


Fig. 3. Clinical spectrum of *SLC26A2* patients.

(Asn425Asp) (both leading to the absence of ST function) cause the intermediate phenotype of DTD.

Our experience on molecular testing for *SLC26A2* (DTDST) mutations in 350 non-Finnish non-Portuguese Caucasian chromosomes show that four common mutations (p.Arg279Trp, p.Arg178X, p.Cys653Ser and IVS1+2T>C or 'Finnish' mutation) account for about 70% of all pathogenic alleles (30, 31), with p.Arg279Trp being present in about 44% of alleles and the other three mutations representing each about 9% of all pathogenic alleles. Although the patients presented here represent only a sample (28 chromosomes) of Portuguese patients with *SLC26A2*-related dysplasias, it is noteworthy that p.Arg279Trp accounted for 64% and p.Arg178Ter for 25%. Thus, a targeted mutation analysis for p.Arg279Trp and p.Arg178Ter in the Portuguese population would be expected to identify approximately 90% of the pathogenic alleles.

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